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[Note]

Measurement of Diffusion Coefficients of Parabens by the Chromatographic Broadening Method

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Summary: The chromatographic broadening method was used for the determination of diffusion coefficients (D) of parabens in water. The D values were obtained with precision, and coefficients of variation were below 0.05. The concentration of methylparaben in the injected solution did not affect D values in the range examined (100 μg/ml–0.5 μg/ml). The D values of parabens decreased with M.W., and that of methylparaben (8.25×10^{-6} cm^2/s, at 25°C, 1.0 μg/ml) was 1.45 times higher than that of *n*-amylparaben (5.70×10^{-6} cm^2/s, at 25°C, 1.0 μg/ml). The D values increased with temperature, and the activation energy for the diffusion of methylparaben and *n*-amylparaben were 18.8 kJ/mol and 20.0 kJ/mol, respectively. The increase of D with temperature could be due mainly to a decrease of viscosity of water.

Keywords: diffusion coefficient; chromatographic broadening method; paraben

Introduction

Mass transport phenomena play a major role in many fields of pharmaceutical sciences. For example, the absorption and distribution of drugs are important in the examination of the pharmacokinetic property, and these could be related to diffusivity of the drugs.[1] Many methods are available for the measurement of diffusion coefficient (D) in many kinds of media.[2–7] The chromatographic broadening method (CBM) is one of them and has been used for determination of D in water and organic solvents.[8, 9] Since a constant solvent flow is required to determine the exact D value, N_2 gas had been used as a power source for the solvent flow in the 1970's.[10, 11] Recently, Mosher measured D of several drugs in buffer and octanol by using a pump for HPLC systems and used the values to examine the interfacial transfer constants.[12] If such pump and UV detector system is applicable for a precise measurement of D values, no special apparatus or labeled drugs would be needed for the measurement.

In this study, the D values of parabens (methyl–amyl) were measured by CBM, using an HPLC apparatus, and their dependencies on concentration (100 μg/ml–0.5 μg/ml) and temperature (17–42°C) were examined.

Materials and Methods

Chemicals

Methyl-, ethyl-, *n*-propyl-, isopropyl-, *n*-butyl-, *sec*-butyl-, isobutyl-, and *n*-amylparaben were purchased from Tokyo Kasei Kogyo. *p*-Aminobenzoic acid was obtained from Wako Pure Chemical (Osaka, Japan).

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Determination of diffusion coefficient

The diffusion coefficients (D) of parabens were determined by CBM. An HPLC system (Shimadzu LC-10A) equipped with 10 m of 0.8 mm i.d. stainless steel tubing (Supelco Inc., PA, USA) was used for the studies. The HPLC system was consisted of pump (LC-10AD), oven (CTO-10AC), detector (SPD-6A), and integrator (CR-5A). Rheodyne 7125 injector with 10 μl quantitative sample loop was used for the sample injection. The stainless steel tubing was placed in the oven and jointed with the injector and the detector directly and was kept at a controlled temperature. Solvent flow was 0.1 ml/min, and the detector was operated at 255 nm. The values of residence time (t_R) and the eluted peak width at half height ($W_{1/2}$) were obtained by the integrator.

The D values were determined from Equation 1, where r is the radius of the capillary tube determined from calibration runs.[12]

$$D = \frac{0.231 \cdot r^2 \cdot t_R}{W_{1/2}^2} \quad (1)$$

p-Aminobenzoic acid, the diffusivity of which is known as 8.43×10^{-6} cm^2/s at 25°C in water,[13] was injected 11 times as the 0.7 mM solution for the calibration runs, and the mean value of r was then calculated to be 0.426 ± 0.003 mm ($\pm$ SD). The obtained r was similar to that in the supplier's specifications of the stainless steel tubing and the value was used for the calculation of D of parabens. The determination of D at each condition was replicated over 11 times and the mean value was shown as the result.

Results and Discussion

The D values of parabens in water are shown in Table I. The values were obtained with precision, and coefficients of variation were below 0.05. The D of methylparaben at

TABLE I. Diffusion Coefficient (D) of Parabens in Water

Parabens	Temp. (°C)	Conc.[a] (μg/ml)	D (cm^2/s) $\times 10^6$	SD $\times 10^6$	n[b]
Methyl-	17	1.0	6.42	0.01	11
	25	0.5	8.44	0.14	11
	25	1.0	8.25	0.30	22
	25	3.0	8.14	0.38	11
	25	10	8.20	0.09	11
	25	100	8.16	0.07	11
	32	1.0	9.65	0.18	11
	37	1.0	10.88	0.14	11
	42	1.0	11.94	0.13	11
Ethyl-	25	1.0	7.48	0.18	11
n-Propyl-	25	1.0	6.81	0.19	16
	37	20	9.06	0.05	11
Isopropyl-	25	1.0	6.94	0.20	16
	37	20	9.09	0.04	11
n-Butyl-	25	1.0	6.31	0.02	11
	25	100	6.53	0.11	11
Isobutyl-	25	1.0	6.40	0.14	11
sec-Butyl-	25	1.0	6.42	0.13	11
n-Amyl-	17	1.0	4.93	0.20	11
	25	1.0	5.70	0.02	11
	32	1.0	7.42	0.37	22
	37	1.0	8.28	0.23	11
	42	1.0	9.24	0.30	11
Isoamyl-	25	2.0	5.74	0.04	11

a) Concentration of the injected solution, *b*) replicated.

25°C was examined at various concentrations (100 μg/ml–0.5 μg/ml). The result shows that D doesn't depend on the concentration of solution injected in the range examined. The D values of parabens decreased with M.W., and that of methylparaben (8.25×10^{-6} cm^2/s, at 25°C, 1.0 μg/ml) was 1.45 times higher than that of n-amylparaben (5.70×10^{-6} cm^2/s, at 25°C, 1.0 μg/ml). In all cases, molecular branching increases D slightly, although no significant differences were found in the values except that of n-amyl-isoamylparaben at 25°C ($p < 0.01$, Student's t-test).[11]

The temperature dependency of D of methylparaben and n-amylparaben was examined from 17°C to 42°C. Those values increase with temperature, and the Arrhenius plot is shown in Fig. 1. The activation energy for the diffusion of methylparaben and n-amylparaben were 18.8 kJ/mol and 20.0 kJ/mol, respectively. Diffusivity for a spherical molecule is related to the temperature and viscosity of the medium as Equation 2, where k is Boltzmann constant, T is absolute temperature, u is mobility, η is viscosity of medium, and r_s is radius of spherical molecule.[1]

$$D = k \cdot T \cdot u = \frac{k \cdot T}{6 \cdot \pi \cdot \eta \cdot r_s} \tag{2}$$

The reciprocal of u is Stokes force, and r_s is also called Stokes-Einstein radius. Since η is viscosity of water and is well known, only r_s is an unknown parameter. If r_s was not changed with temperature, a plot of $u(=D/kT)$ to reciprocal of $6\pi\eta$ should be a straight line as a function of $y = ax$. Thus the value of $1/6\pi\eta$ was calculated from the viscosity of water at each temperature, and the values of u were ploted versus $1/6\pi\eta$. The plots for methylparaben and n-amylparaben are shown in Fig. 2, suggesting that the temperature dependency of r_s is negligible and that of D could be due mainly to the change of water viscosity. The r_s values of methylparaben and n-amylparaben calculated from the corresponding slope of the plot were 3.01×10^{-8} cm and 3.99×10^{-8} cm, respectively, and these values seem reasonable in relation to their molecular sizes.

T diffusivity of drugs should affect the pharmacokinetic and pharmacodynamic properties. However, a direct determination of D values of the drugs in the body should be difficult. To estimate D in some process of the drug disposition, a pharmacokinetic model and computer calculation may be useful. Since this estimated D could be affected by the accuracy of the model and the fitness of the data to the model, an *in vitro* evaluation of D could support to understand the process in the body. CBM is available to evaluate the

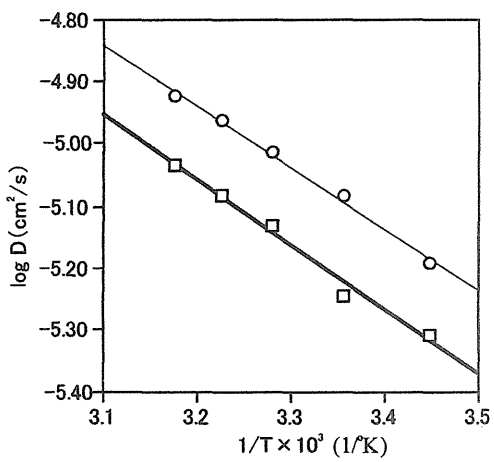

Fig. 1. Arrhenius Polts for D of Methylparaben and n-Amylparaben
Methylparaben (○), n-amylparaben (□).

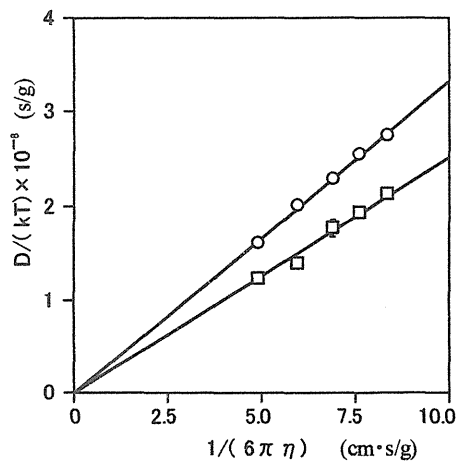

Fig. 2. Relationship between Reciprocal of $6\pi\eta$ and u ($= D/(kT)$)
Methylparaben (○), n-amylparaben (□).

effects of the third components on D of drugs. We are trying to examine the effects of an interaction with biological molecules, such as serum albumin and mucopolysaccharides, on D of drugs by using CBM.[14–16]

In conclusion, the D of parabens in water at several conditions was determined with accuracy by CBM, and the results suggest that CBM is useful for the *in vitro* determination of D. The advantages of CBM as a method for determination of D are as follows: It requires no special apparatus, high concentration (solubility) of drugs in the medium, labeling of the molecule, or long determination time, and D values can be measured in many kinds of solvents that are commonly used for HPLC analysis. Since many solvents and a low-concentration solution of drugs in the solvent are available for the determination of D at varieties of the combination of drugs and solvents, the relationship between D and the physicochemical properties of the drug and solvent can be examined rather simply by CBM.

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